microscopy. We imaged spontaneous and stimulus-induced neuronal activity in the barrel cortex. Transgenic mice exhibited abnormal calcium activity characterized by augmented, long-lasting transients with considerable deviation from the exponential decay. The most evident pathology was observed in response to a repetitive stimulation when subsequent stimuli were presented before relaxation of calcium signal to the baseline. Augmented calcium entry can result from an increase in neuronal spiking or a decrease in a cell buffering capacity. To address the former possibility, we performed *in vivo* electrophysiological recordings of population spiking activity. Our measurements revealed no significant increase in neuronal spiking response compared to age-matched controls. To investigate potential impairment in calcium buffering, we conducted experiments in a neuronal cell line expressing α-synuclein. Inhibitors of the calcium influx into the intracellular calcium stores (mitochondria and endoplasmic reticulum) rescued cytosolic calcium levels, elevated by α-synuclein, while inhibitors of the calcium influx via channels located on the plasma membrane were ineffective. These studies support the notion that α-synuclein dysregulates calcium buffering capacity *in vivo*. Moreover, the characteristic shape of calcium decay and augmentation of the response amplitude during repetitive stimulation can serve as *in vivo* imaging biomarkers for screening of potential therapeutic agents in this model of neurodegeneration.

2156-Plat
SERAF, a Novel Regulator of Store Operated Calcium Entry
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Store operated calcium entry (SOCE) is a principal cellular process by which cells regulate basal calcium, refill intracellular calcium stores and execute a wide range of specialized activities. In recent years tremendous progress has been made in understanding the nature of the molecular components and events that govern SOCE activation. STIM and Orai were identified as the essential components able to reconstitute Ca^{2+} release-activated Ca^{2+} (CRAC) channels that mediate SOCE. Here, we report the molecular identification of SERAF, a novel regulator of SOCE. Using heterologous expression, RNAi-mediated silencing and site directed mutagenesis, combined with electrophysiological, biochemical and imaging techniques; we show that SERAF is an endoplasmic reticulum membrane protein that associates with STIM to facilitate SOCE inactivation following calcium refilling. SERAF, therefore, plays a key role in shaping cytosolic Ca^{2+} signals and determining the content of the major intracellular calcium stores, role that is likely to be important for protecting cells from calcium overfilling.

2157-Plat
Biophysical and Pharmacological Characterisation of Calcium Channels in Human Mast Cells
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The best characterised calcium channel in immune cells is the store-operated calcium release activated calcium channel (CRAC); studies in heterologous expression systems and knockout mice have identified Orai and its regulator STIM1, an ER-resident calcium sensor, as the molecular constituents of CRAC. Members of the TRPC family are also likely to contribute to store-operated calcium entry (SOCE) in immune cells. The expression of Orai and TRPC channels in primary human mast cells has not yet been investigated, but is of key importance given the critical role these cells play in the pathophysiology of type-1 hypersensitivity reactions. Allergic activation occurs following cross-linking of the FcεRI receptor by antigen-IgE complexes that, via PLC-γ signalling, leads to robust activation of SOCE. The resulting calcium signal stimulates secretion and production of pro-inflammatory mediators. The aim of this study was to characterize the biophysical and pharmacological properties of CRAC channels in human lung mast cells (HLMC) following SOCE.
RT-PCR revealed mRNA expression for Orai1, Orai2, STIM1 and STIM2 but not Orai3 in HLMCs. Whole-cell patch clamp recordings of isolated, cultured HLMCs were carried out using a Cs-based internal solution, with added IP_3 and BAPTA to activate SOCE. These conditions activated an inwardly rectifying current with E_{rev}= 18 ± 4mV (n=6), a value less positive than expected for a pure Orai/CRAC current. However, the current was potentiated in divalent-free solution 563.7 ± 6% (n=6), as expected for Orai/CRAC currents, and was significantly attenuated 51.4 ± 8% (n=6), by the Orai-specific inhibitor Synta66 (10μM) (Ng *et al.* 2008., Di Sabatino *et al.* 2009). Calcium imaging in HLMCs loaded with fura-2 AM showed that Synta66 also attenuated anti-IgE induced calcium signaling by 62.2%(n=14). Taken together the data suggest that both Orai and TRPC channels contribute to SOCE in HLMCs.

2158-Plat
Activation of ARC Channels, a Noncapacitative Orai Channel, is Independent of the N-Terminal Domains of STIM1
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The Orai family of Ca^{2+} channels includes the store-operated CRAC channels and store-independent, arachidonic acid (AA)-activated ARC channels. Although both channels are dependent on STIM1 for their activation, they differ in the pool of STIM1 responsible. Physiologically, CRAC channel activation relies on a loss of Ca^{2+} from a luminal N-terminal EF-hand domain of STIM1 resident in the ER membrane. However, ARC channels are exclusively regulated by the pool of STIM1 that is constitutively located in the plasma membrane (PM). Here, the EF-hand is extracellular and is unlikely to ever lose its bound Ca^{2+} - suggesting that STIM1-dependent activation of the ARC channels is very different from that of the CRAC channels.
Expression of just the cytosolic C-terminal region of STIM1 in HEK293 cells resulted in the complete loss of significant CRAC or ARC channel currents following store-depletion or AA addition, respectively. However, attachment of the same STIM1 C-terminus to the inner face of the PM via an N-terminal sequence based on the SH4 domain of Lck, resulted in the full restoration of normal AA-activated ARC channel currents. Store-operated CRAC channel currents, however, were not restored - an effect confirmed in RBL cells, whose endogenous CRAC currents are ~4-5 times larger than those in HEK293 cells. Finally, introduction of a point mutation within the Lck sequence that blocks myristoylation/palmitoylation of this domain, with the consequent loss of PM localization, resulted in the failure of effective ARC channel activation. These data indicate that N-terminal regions of STIM1 (including the EF-hand and SAM domains) play no significant role in AA-dependent activation of the ARC channels, and that the simple location of the C-terminal domain to the cytosolic face of the PM is all that is required.

Platform: Membrane Physical Chemistry II

2159-Plat
Supported Lipid Bilayers on Biocompatible Polysaccharide Multilayers
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Solid-supported phospholipid bilayers are widely used as models to probe the function of cellular membranes and as platforms for membrane protein biosensing applications. Although supported lipid bilayers have a number of advantages in terms of ease of fabrication and characterization, the proximity of the membrane to the solid support is a limitation for applications that involve incorporation of integral membrane proteins. This limitation can be overcome by formation of supported lipid bilayers on soft polymer cushions. We have prepared biocompatible polymer cushions by the layer-by-layer assembly of two polysaccharide polyelectrolytes, chitosan (CHI) and hyaluronic acid (HA), on glass substrates. $(CHI/HA)_5$ films were characterized by atomic force microscopy, giving an average thickness of 57 and roughness of 25 nm in aqueous solution at pH 6.5. Formation of zwitterionic lipid bilayers by the vesicle fusion method was attempted using DOPC vesicles at pH 4 and pH 6.5 on $(CHI/HA)_5$ films. At higher pH adsorbed lipids had low mobility and large immobile lipid fractions; a combination of fluorescence and AFM indicated that this was due to the formation of heterogeneous membranes with defects and pinned lipids, rather than to a layer of surface adsorbed vesicles. By contrast, more uniform bilayers with mobile lipids were produced at pH 4. Fluorescence recovery after photobleaching gave diffusion coefficients that were similar to those for bilayers on PEG cushions and considerably higher than those measured on other polyelectrolyte films. The formation of bilayers containing reconstituted integral membrane protein was compared on PEM and PEG films. These results demonstrate that polysaccharides provide a useful alternative to other polymer cushions, particularly for applications where biocompatibility is important.

2160-Plat
Temperature Dependent FCS Shows Mammalian Cell Membrane is not Poised near Miscibility Transition Temperature
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Immiscibility of liquid mixtures occurs when Gibb's free energy of the separated state is more favorable to that of homogeneous state of the system. Immiscibility is known to be taking action in various lipid mixture membrane systems too. Studies examining macro scale phase separations in ternary lipid mixtures and mammalian membrane blebs have shown that domain separation can also sort membrane components by differential chemical potentials. This behavior is commonly extended to native cell membranes hypothesizing existence of